

## The effect of pretreatment using hydrochloric acid on the characteristics of buffalo hide gelatin

S. Mulyani<sup>1,3</sup>, F. M. C. S. Setyabudi<sup>2</sup>, Y. Pranoto<sup>2</sup> and U. Santoso<sup>2</sup>

<sup>1</sup>Doctoral Program, Faculty of Agricultural Technology, Gadjah Mada University,  
Jl. Flora No.1, Bulaksumur, Yogyakarta 55281 - Indonesia

<sup>2</sup>Faculty of Agricultural Technology, Gadjah Mada University,  
Jl. Flora No.1, Bulaksumur, Yogyakarta 55281 - Indonesia

<sup>3</sup>Permanent Address: Faculty of Animal and Agricultural Sciences, Diponegoro University,  
Tembalang Campus, Semarang 50275 – Indonesia

Corresponding E-mail : [umar\\_s@ugm.ac.id](mailto:umar_s@ugm.ac.id)

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### ABSTRAK

Tujuan penelitian adalah untuk mengidentifikasi variasi konsentrasi asam klorida terhadap *yield* dan sifat fisikokimia gelatin kulit kerbau. Design penelitian menggunakan Rancangan Acak Lengkap, dengan perlakuan variasi konsentrasi asam klorida 0.3M, 0.6M, 0.9 M, 1.2 M dan 1.5M, ulangan sebanyak empat kali, dilanjutkan uji beda nyata terkecil. *Yield*, kekuatan gel, viskositas, pH dan intensitas warna merah gelatin dipengaruhi ( $P<0.05$ ) oleh variasi konsentrasi HCl. *Yield* tertinggi (29.17%) diperoleh pada konsentrasi 0.9M. Kekuatan gel dan viskositas tertinggi pada konsentrasi 1.2M, masing-masing 293.41 g bloom dan 22.17 cP. Gelatin kulit kerbau mempunyai pH 5.08 -5.52. Tingkat kecerahan warna gelatin ( $L^*$ ) 69.92 – 70.97, sedangkan warna merah ( $a^*$ ) 0.55-1.54 dan kuning ( $b^*$ ) 17.54 -19.59. Kadar air gelatin  $7.05\pm0.07$  –  $8.92\pm0.06\%$ , kadar protein  $83.38\pm0.06$  –  $91.11\pm0.03\%$  dan kadar abu  $0.53\pm0.04$  –  $1.23\pm0.09\%$ . Berdasarkan absorpsi spectra fourier transform infrared (FTIR) terdeteksi empat area amida yaitu amida A ( $3402.43$  –  $3448.72$  cm<sup>-1</sup>), amida I ( $1635.65$  cm<sup>-1</sup>), amida II ( $1527.62$  cm<sup>-1</sup>) dan amida III ( $1242.16$  cm<sup>-1</sup>). Variasi konsentrasi HCl mempengaruhi *yield*, karakteristik dan struktur sekunder gelatin kulit kerbau. Konsentrasi 0.9M merupakan kondisi optimum untuk memperoleh *yield* tertinggi. Karakteristik gelatin kulit kerbau dengan pretreatment menggunakan asam klorida telah memenuhi standar kualitas Gelatin Manufacturing Institute of America (2012) .

*Kata kunci : asam klorida; gelatin , kulit kerbau, pretreatment, yield*

### ABSTRACT

The objective of the research was to identify the effect of the concentration of hydrochloric acid (HCl) on yield and the characteristics of gelatin from buffalo hide. A completely randomized design was used with various concentrations of HCl treatment 0.3M, 0.6M, 0.9 M, 1.2 M and 1.5M, and four times replication, followed by least significant difference test. The HCl concentration gave significantly effect ( $P<0.05$ ) on yield, viscosity, pH, gel strength and redness of gelatin. The highest yield (29.17%) was obtained at a concentration of HCl 0.9M. The highest gel strength and viscosity at concentration of 1.2M, were 293.41g bloom and 22.17 cp, respectively. The pH of the gelatin were 5.08 to 5.52. The lightness level ( $L^*$ ) was 69.92-70.97, whereas the redness ranged ( $a^*$ ) from 0.55 to 1.54 and the yellowness ranged

(b\*) from 17.54 to 19.59. The moisture, protein and ash concentrations of gelatin were  $7.05 \pm 0.07$ - $8.92 \pm 0.06\%$ , protein content of  $83.38 \pm 0.06$  - $91.11 \pm 0.03\%$ , and the ash content  $0.53 \pm 0.04$  to  $1.23 \pm 0.09\%$ , respectively. Based on the spectra absorption of infrared were detected four areas amides: amide A ( $3402.43$ - $3448.72$  cm<sup>-1</sup>), amide I ( $1635.65$  cm<sup>-1</sup>), amide II ( $1527.62$  cm<sup>-1</sup>) and amide III ( $1242.16$  cm<sup>-1</sup>). The differences in the concentration of HCl had impact on yield, characteristics and secondary structure of buffalo hide gelatin. The HCl concentration at 0.9M was the optimum condition to obtain the highest yield. The characteristics of buffalo hide gelatin after pretreatment using hydrochloric acid had met the standards of GMIA (2012) .

**Keywords:** *Hydrochloric acid, gelatin, buffalo hide, pretreatment, yield*

## INTRODUCTION

Buffalo is the one of tropical livestock producing skin by-product that may be used as raw material for producing gelatin. The skin by-product of buffalo had thick and strong structure because slaughtering age buffalo was older than slaughtering age of cows and commonly used as working livestock (Huda *et al.*, 2011). The thickness of skin by-product is 6-8 mm with approximately 11.5 % of the animal live weight (Spanghero *et al.*, 2004). The thickness and firmness of skin by-product indicated. A high collagen fibers. A high collagen of the hide is supported by the high pyrrolidine group of amino acid hydroxyproline.. The pyrrolidine group attributes the collagen with a high thermal stability (Haugh and Draget, 2009), so heat extraction only could not optimize the extraction process. Therefore, acid pretreatment process is required to optimize the extraction process (Niu *et al.*, 2013).

The acid pretreatment results in increasing weak intermolecular and intra- molecular structural bond in the skin collagen protein because of partial breakdown of the amino acid bond chain (Kolodziejska *et al.*, 2007). This condition is used in the extraction process resulting in collagen solubility (Johnston-Banks, 1990). Acid pretreatment at different acid concentrations has an impact on yield and gel strength (Ahmed and Benjakul, 2011; Nikoo *et al.*, 2014). An acid helps the release and dissolve of some proteins, fat and other components of collagen. This also disturbs cross-interaction molecules of the collagen thereby increasing the efficiency of the extraction. Hydrochloric acid is a type of strong acid that could be dissociated perfectly, thus producing many hydrogen ions to break crosslinks in collagen molecules. Hydrochloric acid requires lower concentration to achieve an optimal extraction condition compared to other type of acid (Niu *et al.*, 2013). More

higher acid concentration may cause over-hydrolysis of collagen molecules and losing protein during washing process (Binsi *et al.*, 2009; Jamilah and Harvinder, 2002). The hydrolysis rate of the collagen molecules influences the yield and characteristics of gelatin. Therefore, the optimization of the acid concentration prior to the extraction is required to avoid over-hydrolysis. This study was aimed to clarify the effect of the concentration of the HCl concentration on the gelatin yield and the characteristics of buffalo hide.

## MATERIALS AND METHODS

### The extraction of buffalo hide gelatin

Wet salt preserved buffalo hide was obtained from CV. Panji Jaya in Cegoroyoso village of Pleret sub district of Bantul, Yogyakarta with the criteria of male buffalo of 2-3 years. The hide washed using tap water and then the hide was soaked in 2% (w/v) camphor solution till the hair was completely removed. Subsequently, the processed hide was scratched to remove the hair and the fat and then was washed using tap water till its pH was 7-7.5. After the removal of the hair and the fat, the processed hide was kept frozen at -18° C (Said *et al.*, 2011). Before the pretreatment, it was thawed at 4°C for 20 hours and cut into small pieces (1x1cm) (Ktari *et al.*, 2014).

The gelatin from buffalo hide was extracted according to the method of Niu *et al.* (2013) with modification. The pieces of the buffalo hide were soaked in 0.5M NaOH (1:4;w/v) for 2 hours. Subsequently, the hide was soaked in HCl at the concentrations of 0.3M, 0.6M, 0.9M, 1.2M and 1.5M (1:4;w/v). The processed hide was drained and washed six times till reaching the pH 5-6. The pretreated hide was extracted with distilled water (1:4;w/v) at 65°C in water bath (Memmert WNB7-45 type) for 5 hours and then followed in similar manner at 70°C for the second step extraction. The extracting result was roughly

sieved and dried using cabinet drier at 50-55°C for 48 hours. The extracted gelatin yield was calculated using formula of Ktari *et al.* (2014):

$$\text{Yield (\%)} = \frac{\text{Dry gelatin weight}}{\text{Fresh hide weight}} \times 100$$

### Proximate Analysis of Hide and Gelatin

The moisture, protein, and ash contents of the buffalo hide and gelatin were determined using proximate analysis following AOAC guideline (2005). Analysis of each proximate was done in three replication.

### Determination of Hydroxyproline

A hundred milligram samples of skin or gelatin was crushed and hydrolyzed by adding 5ml 6N HCl for 12 hours at 110°C in dry-bath. Then, NaOH 6 N was used to neutralize the samples. Two ml acetate or citrate buffer and 0.3 M NaCl were added till 25 ml volume was reached. The solution was transferred to the 300 µl isopropanol and oxidant solution containing testing flask. At this time the mixture was quickly mixed for 4 minutes, and then was added 4 ml of Ehrlich's solution in 3 ml of 60% acid per chloride (v/v). The solution mixture was shake for 25 minutes in water bath (60°C). The optical density of solution mixture was measured using a spectrophotometer at wavelength of 660 nm. The calculation of hydroxyproline content was followed by standard hydroxyproline solution curve (Sigma Chemical).

### Determination of Gel Strength

Gelatin powder (6.67 g) was dissolved in 100 mL of distilled water (6.67%; w/v) and was stirred using magnetic stirrer and then heated at 60°C for 15 min and incubated 16-18 hours at 10°C. Subsequently, it was measured using texture analyzer TA-XT plus HD (Stable Micro System Ltd., UK) at the probe speed of 0.5 mm/sec and 4 mm depth (Benjakul, *et al.*, 2009).

### Determination of Acidity (pH)

Zero point two gram sample was weighted, dispersed into 20 ml of distilled water at 80°C, and homogenized using magnetic stirrer. The acidity of solution was measured using pH meter (British Standard 757).

### Measurement of Color

Chromameter (Konika Minolta Sensing, INC, Japan) with the Hunter system and

expressed in L, a, and b value was used to measure the color of gelatin. The L value for the lightness were in the range of 100 = white and 0 = black. The a values were in the range of -50 (green) and +50 (red). The b values were in the range -50 (blue) and (yellow) (Jamilah and Harvinder, 2002).

### Determination of Fourier Transform Infrared (FTIR) Spectroscopy

Gelatin powder (2 mg) was made pieces in 100 mg of kalium bromide (KBr). The sample strip was read through FTIR (Shimadzu PC-8201) in the range of wave number 4000-650 cm<sup>-1</sup> (Kaewruang *et al.*, 2013).

### Statistical Analysis

This study was designed using complete random design with various HCl concentration as treatments with four replicates. The Analysis of variance and least significant difference test (Steel and Torri, 1980) were performed using SPSS 20.0 for Windows. Especially for proximate, hydroxyproline and FTIR were determined using descriptive analysis based on the average mean with three times replicates.

## RESULTS AND DISCUSSIONS

### Yield

Yield is often related to the extraction efficiency in gelatin production. The increasing high yield is indicative of production process efficiency (Kolodziejska *et al.*, 2007). The gelatin extraction yield at various HCl concentrations in the pretreatment process are shown in Table 1. The values were in the range of 14.67% to 29.17%, with the highest yield at the HCl concentration of 0.9 M. The values decreased to 25.59% and 24.53%, when the HCl concentration was increased up to 1.2 and 1.5 M.

The HCl concentrations gave significantly affect ( $P < 0.05$ ) on the yield of buffalo hide gelatin. Acid pretreatment is required as H<sup>+</sup> ion provider that plays an important role in breaking both intra and intermolecular bonds of collagen, so collagen is easily dissolve in water. The increasing acid concentration was followed by the increased H<sup>+</sup> concentration in solution, finally the collagen hydrolysis process was accelerated. The higher hydrolysis rate caused the triple helix collagen breakdown into more bigger α, β and γ chains. In other words the collagen conversion into gelatin increased in accordance with the

Table 1. Yield and the Characteristics of Buffalo Hide Gelatin by Pretreatment Using HCl

Concentration of HCL	Yield* (%)	Gel Strength (g bloom)*	Viscosity* (cP)	pH*	Measurement of Color		
					L <sup>ns</sup>	a <sup>*</sup>	b <sup>ns</sup>
0.3 M	14.67±1.40 <sup>c</sup>	265.08±3.64 <sup>b</sup>	18.65±1.20 <sup>ab</sup>	5.52±0.03 <sup>a</sup>	69.92±1.94	0.55±0.22 <sup>a</sup>	17.54±0.59
0.6 M	26.44±2.10 <sup>b</sup>	239.44±13.45 <sup>c</sup>	17.92±1.20 <sup>b</sup>	5.16±0.02 <sup>b</sup>	70.97±0.91	1.54±0.19 <sup>b</sup>	18.90±0.58
0.9 M	29.17±2.10 <sup>a</sup>	242.35±12.90 <sup>c</sup>	16.37±1.50 <sup>b</sup>	5.15±0.05 <sup>bc</sup>	70.66±1.46	1.51±0.13 <sup>b</sup>	18.14±1.52
1.2 M	25.59±0.80 <sup>b</sup>	293.41±7.30 <sup>a</sup>	22.17±1.70 <sup>a</sup>	5.09±0.04 <sup>c</sup>	70.55±0.47	1.21±0.56 <sup>b</sup>	19.59±1.80
1.5 M	24.53±0.90 <sup>b</sup>	248.94±13.45 <sup>bc</sup>	18.23±2.80 <sup>ab</sup>	5.08±0.03 <sup>c</sup>	70.82±0.83	1.27±0.98 <sup>b</sup>	17.87±1.24

\* Means within the same column followed by different superscript are significant different (P<0.05); ns = non-significant (P>0.05). L: lightness, a: redness, b; yellowness

increasing yield value. On the contrary, the increase in the HCl concentration more than 0.9M resulted in the decrease in the yield. Continuing hydrolysis process may cause more shorter collagen molecule chain and hence there were many dissolved collagen in the washing process. Once an optimum condition has been met, the increase in the acid concentration would decrease the gelatin yield because of the over-hydrolysis of the collagen and there were many proteins lost in the washing process (Niu *et al.*, 2013; Jamilah and Harvinder, 2002).

### Chemical Composition of Hide and Gelatin

The composition of the hide and the gelatin from buffalo was represented in the Table 2. The raw material of the wet salt preserved buffalo hide has gone through the process of soaking, so that it looked like fresh skin. Said *et al.* (2011) reported that fresh skin contained 64% moisture, 33% protein and 0.2% mineral. Alfaro *et al.* (2014) suggested that the protein content in the hide described maximum collagen content found in skin tissue.

In general, the hydroxyproline content of the buffalo gelatin was lower than the hidroxypoline buffalo hide (% dry matter). The collagen conversion process into gelatin was carried out through pretreatment and extraction process at 65-70°C so that the hydrolysis process caused the decrease in the hydroxyproline content.

The moisture content of gelatin tended to diminish by the increasing HCl concentration used in the pretreatment process. The acid treatment brokedown both the intra- and intermolecular collagen crosslink so that the

collagen structure became more open and weak. The water holding capacity lessened and increased the quantity of water evaporation during drying process (Sompie *et al.* 2015). The moisture of buffalo hide gelatin ranged from 7.05 ± 0.07 to 8.92 ± 0.06%. These value were lower than the results of Alfaro *et al.* (2014) and standard commercial gelatin is 9-14% (Eastoe and Leach, 1977).

The protein content of gelatin directly related to the gel strength and viscosity (Said *et al.*, 2011). The protein content of buffalo hide gelatin were in the range of 83.38 ± 0.06 to 91.11 ± 0.03%. (Table 2). This result was almost consistent with other studies. Said *et al.* (2011) examined the gelatin of goat skin, the protein content were in the range of 89.37 ± 1.19% - 90.74 ± 1.82%. Sompie *et al.* (2015) reported that gelatin from pig skin, were in the range of 86.03 - 89.22%. This result was also similar to commercial gelatin approximately at 89.63% (Pranoto *et al.*, 2006). The collagen dissolving rate is influenced by the material and time used for the pretreatment process (Wang *et al.*, 2008; Said *et al.*, 2011). The protein content of gelatin from buffalo hide increased from 0.3M to 0.6 M and 0.9 M. The increase in HCl concentration resulted more broken crosslink of both intra and intermolecular collagen that there are many proteins dissolved in the extraction process. The big number of the dissolved protein caused the increase in the protein content of gelatin. At the concentration of 1.2 M and 1.5 M the protein content did not further increase because the optimum concentration has been met. The increase in the acid concentration would

Table 2. Chemical Composition of Hide and Gelatin from Buffalo by Pretreatment Using HCl

Composition (%)	Buffalo Hide	Buffalo Hide Gelatin				
		HCl 0.3M	HCl 0.6M	HCl 0.9M	HCl 1.2M	HCl 1.5M
Moisture	68.62±0.13	8.92±0.06	7.41±0.05	7.09±0.01	7.05±0.08	7.64±0.21
Protein	30.22±0.06	89.35±0.01	83.38±0.06	91.11±0.03	90.93±0.01	90.89±0.03
Ash	0.72±0.01	1.23±0.09	0.87±0.07	0.56±0.01	0.59±0.04	0.53±0.04
Hydroxy-prolin	0.25±0.04	2.18±0.02	0.71±0.02	0.44±0.01	0.46±0.01	0.46±0.02

Results are means ± standard deviation (n=3)

result in over-hydrolysis, therefore many proteins lost during washing (Niu *et al.*, 2013; Sompie *et al.*, 2015).

The gelatin extract from buffalo hide had the ash content approximately from  $0.53 \pm 0.04$  to  $1.23 \pm 0.09\%$ . These values met GMIA standard, ranging from 0.3 - 2.0% and the maximum value is 2.6% recommended by Muyonga *et al.* (2004). The highest ash content found in the treatment at the HCl concentration of 0.3 M, and then decreasing at the HCl concentration of 0.6M and 0.9M. Low ash content is indicative of good quality gelatin extraction process (Uriarte *et al.*, 2011; Chandra and Shamasundar *et al.*, 2015).

### Gel Strength

Gel strength is a key parameter in the determination of gelatin quality. The gel strength indicates the capability of gelatin to change from gel phase into sol phase and vice versa (Kusumawati *et al.*, 2008). The value of buffalo hide gelatin at the HCl concentration of 0.3 M to 1.5 M was in the range of 239.44 - 293.41 g bloom.

The results of the measurement of the gel strength described that the HCl concentrations had significant impact ( $P < 0.05$ ) on the gel strength (Table 1). The HCl concentration of 1.2 M showed the highest gel strength value ( $293.41 \pm 0.73$  g bloom), while the gel strength value at the HCl concentration of 1.5 M decreased to the same value those found at the HCl concentrations of 0.9 and 0.6 M. The concentration of pretreatment material had impact on the gelatin gel strength. High concentration of acid could cause a decrease in the gel strength (Ockerman and Hansen, 2000). At the concentration of 1.2 M, HCl broke down amino acid polymer chain at the right limit. This

improved the effect in formation process of gel. Subsequently, amino acid monomer chains joined to form a continuous three-dimensional structures and bond water to form compact gel structure. The difference in the gelatin gel strength related to amino acid chain length, imino acid composition (prolin and hydroxyprolin), the concentration of gelatin and the distribution of molecule weight (Arsenen and Gildberg, 2007; Karim and Bhat, 2009). Gelatin with long chains had high gel strength values. The values have met the GMIA standard (2012), which were 50-300 g bloom to be applicable in general industry.

### Degree of acidity (pH)

The HCl concentration significantly effected ( $P < 0.05$ ) on gelatin acidity (Table 1). The Increasing HCl concentration, decreased in pH of gelatin, at 1.2 M and 1.5M of HCl concentration, the pH was unaffected significantly. The higher HCl concentration caused a progressive increase in the number of hydrogen ions (Kusumawati *et al.*, 2008). The pH value at the HCl concentrations of 0.3 M to 1.5 M was in the range of 5.08 – 5.52. It still met industrial standard (GMIA, 2012), which were 3.8 – 6.0.

### Viscosity

The viscosity of the buffalo hide gelatin were in the range of 16.37 to 22.17 cp. The values have met GMIA quality standard (2012) for food industry, which were 1.5 – 7.5 cp. The HCl concentration gave significantly effect ( $P < 0.05$ ) on the viscosity. One of the influencing factors of the gelatin viscosity is pH value (Kusumawati *et al.*, 2008). Considering the pH values summarized in Table 1, the pH of the buffalo hide gelatin was in the range that still met industrial quality standard and optimal pH for gelatin production.

Jamilah and Harvinder (2002) suggested that the viscosity of the gelatin could be increased by extracting the gelatin at the pH of 3-10.5. In the range of the optimum pH for collagen hydrolysis (the breaking of the collagen chain took place in limit of polypeptide) the protein chains are still long and the gelatin viscosity was high (Said *et al.*, 2011; Pelu *et al.*, 1998).

### Color

The color and the lightness of the gelatin present esthetic characteristics influencing the acceptability and the applicability of the gelatin. In general, the color of the gelatin did not have any significant impact on its function (Table 1). However, more light colors are more liked because they provided more flexibility to be mixed into certain food system (Rahman and Jamalulain, 2012; Shyni *et al.*, 2014). The results of the measurement of the colors of the buffalo hide gelatin were expressed in brightness values ( $L^*$ ), redness intensity ( $a^*$ ) and yellowness intensity ( $b^*$ ). The HCl concentration did not have any significant impact ( $P>0.05$ ) on the brightness value or on the yellowness intensity, but it had significant impact ( $P<0.05$ ) on the intensity of the red color of the gelatin. The use of the HCl concentrations of 0.3 – 1.5 M was still in the safe limit for pretreatment process. This means that though the hydrolysis process proceeding higher with the increase in the concentration of  $H^+$

(more higher HCl concentration), this was still in the expected limit. There was an increase in the red color intensity at 0.6 M of HCl concentration as compared to 0.3 M, but this was not significantly different ( $P>0.05$ ) with 0.9 M, 1.2M, and 1.5M. The higher collagen hydrolysis caused the increasing supply of free amino group. The amino group reacts to the skin carbonyl compound is non- enzymatic browning reaction this cause the increase in the redness and yellowness of the gelatin (Nagarajan *et al.*, 2012; Sae-Law and Benjakul, 2015; Sae-Law *et al.*, 2016).

### Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR is applied to observe the change in the functional group and the secondary structure of the gelatin (Muyonga *et al.*, 2004; Kaewruang *et al.*, 2013). Figure 1 shows the FTIR spectra of buffalo hide gelatin through the pretreatment with HCl at the various concentrations from 0.3 to 1.5M. Muyonga *et al.*, (2004) reported that there were four identified amide areas in the wave numbers of 3600 – 2300 per cm (amide A), 1656 – 1644 per cm (amide I), 1560-1335 per cm (amide II) and 1240 – 670 per cm (amide III).

The amide A of the buffalo hide gelatin at the HCl concentrations of 0.3 M, 0.6 M, 0.9 M, 1.2 M and 1.5 M were respectively detected in the

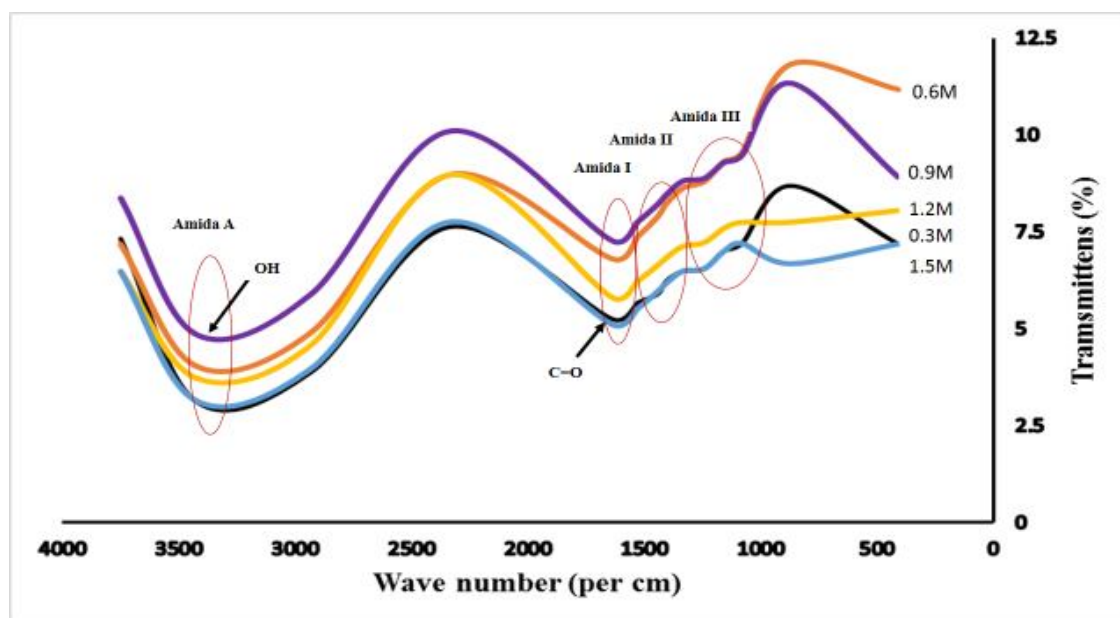


Figure 1. FTIR Spectra of Buffalo Hide Gelatin by Pretreatment using HCl

wave numbers of 3402.43 per cm, 3425.58 per cm, 3425.58 per cm, 3448.72 per cm and 3425.58 per cm. The peak of the amide A related to the stretch of the group N-H and was indicative of the existence of hydrogen bond. Vibration normally take place in the wave numbers of 3400 – 3440 per cm. The lower wave numbers indicated bigger molecular degradation of the gelatin. The degradation produces free amino group that interacts with other reactive groups (Kaewruang *et al.*, 2013). The gelatin at the HCl concentration of 1.2M had amide A peak with the highest wave number (Figure 1). In other words, the molecular degradation was the smallest with the longest molecular chain that the viscosity value and the strength of the gel were the highest.

The amide I presents the stretch C=O or hydrogen bond in a pair with COO. The absorbing area of the amide I is often used to analyze protein secondary structure (Nagarajan *et al.*, 2012). The amide I of the buffalo hide gelatin at the HCl concentrations of 0.3 M, 0.6 M, 0.9 M, 1.2 M and 1.5 M, respectively were detected in the same wave number of 1635.64 per cm. Yakimates *et al.* (2005), Nagarajan *et al.* (2012) stated may represent the peak absorption took place in the wave number of 1633 per cm representing the characteristic of the coil structure of the gelatin. Concerning with transmission intensity, the gelatin at the highest HCl concentration of 0.9 M experienced triple helix dispersal into coil structure whose quantity was the highest. Therefore, the pretreatment with the HCl at the concentration of 0.9 M produced the highest yield (Table 1).

The amide II of the buffalo hide gelatin at the HCl concentrations of 0.3 M, 0.6 M, 0.9 M, 1.2 M, and 1.5 M was detected in the range of the wave numbers of 1527.62 – 1342.46 per cm (Figure 1). The absorption of the amida II takes place because of the vibration of the N-H group and the stretch of the group C-N (Nagarajan *et al.*, 2012). The amide III was detected in the wave numbers of 1242.16 – 871.82 per cm. This showed the combination of the stretch peak of C-N and the deformation of N-H in the amide was observed as the absorbance of the group CH<sub>2</sub> of the glycine backbone chain and the proline side chain. The gelatin at the HCl concentration of 0.9 M was indicative of the highest transmittance intensity for both amide II and III. This indicated that the bigger random molecular structure may be exist because the a-

helix transformation into coil random structure may take place more frequently with the increase in the acid concentration of 0.9 M. The change related to the loss of the triple helix structure as a result of collagen denaturizing into gelatin (Muyonga *et al.*, 2004; Nagarajan *et al.*, 2012).

## CONCLUSION

The difference in HCl concentrations had impact on the yield, the characteristics and the secondary structure of the buffalo hide gelatin. The HCl concentration of 0.9 M represented the optimum concentration to produce the highest yield. In general, the characteristics of the buffalo hide gelatin with the pretreatment process using HCL had the characteristics that met the GMIA standard.

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